

## Effect of Decontamination Agents on the Microbial Population, Sensorial Quality, and Nutrient Content of Grated Carrots (*Daucus carota* L.)

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Several decontamination agents including water, sodium hypochlorite, peroxyacetic acid, neutral electrolyzed oxidizing water, and chlorine dioxide gas were tested for their effectiveness to reduce the natural microflora on grated carrots. Microbial reductions of the total aerobic count obtained after the different treatments varied between 0.11 and 3.29 log colony-forming units (cfu)/g. Whether or not a decontamination step induced significant changes in the sensory attributes of grated carrots is highly dependent on the type and concentration of disinfectant. To maintain the nutritional value, the influence of the decontamination agents on carotenoid content,  $\alpha$ -tocopherol content, total phenols, and antioxidant capacity was studied. Besides the part of the nutrients that was leached away from the cutting areas by water, the nutrient losses caused by adding sanitizers were rather limited. Compared with the untreated carrots  $\alpha$ -tocopherol content was, however, significantly reduced when 250 ppm of peroxyacetic acid (–80%) or 200 ppm of sodium hypochlorite (–59%) was used. Additional losses in carotenoid content were caused by contact with chlorine dioxide gas (–9%). On the condition of an optimized decontamination process toward time and concentration, the microbial quality of fresh-cut carrots could be improved without negatively influencing their sensory quality and nutrient content.

**KEYWORDS:** Disinfection; fresh-cut; microbiology; sensory analysis; nutrient content; *Daucus carota* L.

### INTRODUCTION

Over the past decade consumers have become more and more health-conscious and aware of the close correlation between nutrition and health. This fact in combination with a demanding lifestyle resulted in an increasing demand for fresh, healthier convenience-type of foods such as fresh-cut vegetables (1). The fresh-cut produce industry, in turn, meets this demand by offering a wide spectrum of value-added, conveniently packaged, ready-to-eat vegetables.

There are several steps in the fresh-cut produce chain and therefore many points for potential microbial contamination (2). Nevertheless, there is no step included during processing that reduces the predominant microbial flora, except the washing treatments. As a result, fresh-cut produce has been associated with foodborne illnesses caused by pathogenic microorganisms including *Escherichia coli*, *Salmonella* spp., *Listeria monocytogenes*, and Noroviruses (3). To increase the safety of fresh-cut produce, decontamination agents such as sodium hypochlorite are often used during the washing procedure. Due to a wide range of limitations associated with the use of sodium hypochlorite, such as the limited efficiency in the presence of organic

material, the antimicrobial action in a narrow pH range (4), the corrosion of processing equipment, the liberation of chlorine vapors during production, and the formation of harmful disinfection byproducts (5, 6), alternative agents such as peroxyacetic acid, neutral electrolyzed oxidizing water, and gaseous chlorine dioxide were suggested. Until now, most of the research on fresh-cut carrots was performed on the survival of artificially inoculated pathogens such as *L. monocytogenes*, *E. coli* and *Salmonella* after treatment with gaseous chlorine dioxide (7), sodium hypochlorite (8–10), and peroxyacetic acid (10, 11). Only a few papers deal with the effect of these sanitizers on the initial microbial count as well as during storage life of minimally processed carrots (12, 13).

Furthermore, vegetables in general and in particular carrots are a rich source of essential nutritional components such as vitamins and secondary plant metabolites. These phytochemicals exhibit a protective effect against deleterious reactive oxygen species and radicals, which has been associated with a reduced risk of age-related diseases such as cancer and cardiovascular diseases (14, 15). More specifically, carrots are widely known as the major source of carotenoids ( $\alpha$ - and  $\beta$ -carotene) (16) and contain also other antioxidants such as polyphenols and a minor amount of  $\alpha$ -tocopherol and vitamin C. Although the health-promoting effects of vegetables are generally accepted, the average intake of fruits and vegetables is still below the

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recommendations of the WHO (400 g/person/day) (17). The offer of a broad range of minimally processed vegetables makes it possible to meet those recommendations and to balance the diet in a convenient way on the condition that processing steps and postharvest treatments such as the use of a decontamination agent during washing do not cause a significant reduction of their nutrient content.

The aim of the present study was to test different sanitizers, that is, water, sodium hypochlorite, electrolyzed oxidizing water, peroxyacetic acid, and gaseous chlorine dioxide, for their decontamination efficiency toward the initial microbial load as well as for their effect on the sensorial quality of minimally processed carrots. Furthermore, the effect of these decontamination treatments on the nutritional quality of minimally processed carrots, more specifically on their carotenoid, total phenol, and vitamin E contents, and on the antioxidant capacity was evaluated.

## MATERIALS AND METHODS

**Plant Material.** Carrots (*Daucus carota* L.) were obtained from a local wholesale business. They were transported to the laboratory within 30 min. On their arrival the carrots were immediately manually processed. The carrots were peeled and grated in sticks of 0.15 × 0.15 × 3.5 cm (Compacto Kitchen Cutter, Philips, Eindhoven, The Netherlands). These sticks correspond with the ones traditionally sold in the market. Because of practical limitations, different carrot batches were bought to test the different decontamination agents.

**Reagents.** Ethanol (pa), methanol (HPLC grade and pa), petroleum ether (HPLC grade), ethyl acetate (HPLC grade), acetonitrile (HPLC grade), chloroform (pa), sodium sulfate (anhydrous), sodium chloride (pa), sodium acetate·3aq (pa), and acetic acid (pa) were purchased from Chemlab (Zedelgem, Belgium). Magnesium carbonate, 2,6-Di-*tert*-butyl-4-methylphenol (pa) (BHT) and oxalic acid dehydrate (99%, pa.) were obtained from Acros Organics (Geel, Belgium).  $\beta$ -Carotene ( $\geq 95\%$ , HPLC), lutein, *trans*- $\beta$ -apo-8'-carotenal,  $\alpha$ -tocopherol (synthetic,  $\sim 95\%$ , HPLC),  $\gamma$ -tocopherol ( $\geq 96\%$ , HPLC), gallic acid, Folin-Ciocalteu's phenol reagent (2 N, with respect to acid), 2,4,6-tris(2-pyridyl)-s-triazine (TPTZ,  $\geq 99.0\%$ , TLC), iron(II) sulfate heptahydrate (pa., ACS reagent,  $\geq 99.5\%$ , RT), and sodium hypochlorite ( $\geq 4\%$ , reagent grade) were purchased from Sigma-Aldrich (Steinheim, Germany). Triethylamine and isopropanol (HPLC-grade) were obtained from Merck (Darmstadt, Germany). Hexane (pa and HPLC grade), sodium carbonate (pa), NaCl, and hydrochloric acid (37%, pa) were obtained from VWR (Fontenay Sous Bois, France). Peroxyacetic acid (Chriox 5) was obtained from Christeyns NV (Ghent, Belgium). Chlorine dioxide stock solution was delivered by Vernagene (Bolton, U.K.).

**Decontamination Treatments.** A mass of 100 g of carrots was immersed in 1 L of disinfectant solution at room temperature under continuous agitation (150 rpm) on an orbital shaker (Ika, Staufen, Germany) for 1 or 5 min depending on the experiment. Afterward, the excess of surface water or disinfectant solution was removed by means of a manual kitchen centrifuge (Zyliss, Bern, Switzerland).

The produce was treated with different disinfectant solutions: water, sodium hypochlorite, electrolyzed oxidizing water (EOW), commercially available peroxyacetic acid, and gaseous chlorine dioxide. The control series were carrots that were peeled and grated. Peeled and grated carrots rinsed with potable water were used as reference treatment. Starting from an aqueous sodium hypochlorite solution and after performing an iodometric titration in acidic environment, solutions with the appropriate concentrations (20 and 200 mg free chlorine/L) were made. Finally, the pH was adjusted to pH 6.00 with hydrochloric acid.

On a daily basis the concentration of the commercially available peroxyacetic acid stock solution was determined by an iodometric titration. Furthermore, dilutions of 80 and 250 mg/L were made of which the pH was, respectively, 5.7 and 4.4.

Neutral electrolyzed oxidizing water (NEW) was generated using an Ecodis 0.20.2-4A/2 (Ecodis NV, Schoten, Belgium). It mainly contains a vessel with water or diluted NaCl solution, a pump (IWAKI,

Tokyo, Japan), a flow meter (SK 50, Georg Fisher, Brussels, Belgium), a control unit, and an electrolytic cell with two anodes and one cathode without a separating membrane. By alternately varying the NaCl concentration in the starting solution, the flow rate, and the voltage or the current over the electrodes, NEW with different free chlorine concentrations was produced. The free chlorine concentration in NEW was determined according to the *N,N'*-diethyl-*p*-phenylenediamine (DPD) method by means of a single-ion portable photometer (HI 93711, Hanna Instruments, Woonsocket, RI). To produce NEW with 3 mg/L of free chlorine (pH 7.50) the electrolytic cell was fed with 15 L/h of potable water and the current over the electrolytic cell was 0.7 A. A NaCl solution of 0.1% (w/v) combined with a flow rate of 20 L/h and a current of 1.3 A over the electrolytic cell was necessary to obtain water with 30 mg/L of free chlorine (pH 7.87).

The gaseous chlorine dioxide treatment system contained a vacuum pump (N.811 KN18, KNF Neuberger, Freiburg, Germany) of which the suction side was used as discharging side and the discharging side as suction side, a purging bottle (1 L) with a sintered glass frit (porosity 1, diameter = 20 mm), a flow meter (A6100, ABB, Zurich, Switzerland) with needle valve, a treatment chamber, and a sampling device. The lockable treatment chamber made of stainless steel had dimensions of 35 cm × 35 cm × 35 cm. A perforated (3 mm diameter, 2 mm between the perforations) stainless steel drum with a volume of about 21.2 L was tumbling inside the treatment chamber. A lockable entrance (10 cm × 20 cm) was foreseen on the drum to bring the sample aseptically into the drum. On the interior side of the drum three paddles of 4–28 cm were attached. The drum was driven by the motor of a stirrer (RZR 50, Heidolph, Nürnberg, Germany) with a speed of 45 rpm. The gaseous chlorine dioxide entered the cabinet at the bottom. The temperature and the relative humidity inside the chamber were measured with a thermohygrometer (Digitron 2020R, Devon, U.K.) through a ball valve connected at the right side of the treatment chamber. The gaseous chlorine dioxide entered the cabinet centrally at the bottom. The homogeneous distribution of the chlorine dioxide gas was realized by the combined action of the blowing of a fan (12 V, 0.7 A) placed 5 cm above the inlet for the gas and the mixing activity of the rotating drum and the up and down movement of the fresh-cut produce caused by the presence of the blades. The different parts were joined with silicon tubing with an internal diameter of 8 mm and a thickness of 2 mm.

To treat the fresh-cut produce 2 kg of grated carrots was brought into the drum with an adjusted relative humidity of approximately 90%. A 1000 mg/L solution of chlorine dioxide was prepared and heated to 48 °C in a hot water bath. Subsequently, the chlorine dioxide gas was stripped from the solution by air bubbling (6 L/h). This liberated gas was guided through the flow meter toward the inlet for the cabinet. Treatment was achieved at room temperature and took 10 min in which a stripping phase of 30 s was included. At regular time intervals gas samples were taken with the sampling device connected to the back side of the cabinet to monitor the chlorine dioxide gas concentration in the cabinet. When the treatment was finished, the cabinet was opened and samples were taken for analysis. Before and after the treatment, the whole equipment was cleaned and disinfected with ethanol. Because of safety concerns the treatment chamber was placed in a safety hood.

To determine the chlorine dioxide concentration in solution, an iodometric titration was performed. To monitor the gaseous chlorine dioxide concentration in the treatment chamber, samples were taken by means of an air sampling pump (Gylair 3, Sensidyne, Clearwater, FL) during 15 s at 1 L/min. Two impingers with 20 mL of KI (7%, w/v) in buffer (pH 7) were serially placed between the treatment chamber and the air sampling pump. In this way the air sample was scrubbed and the chlorine dioxide was neutralized. After the content of the impingers had been quantitatively transferred to an Erlenmeyer flask and hydrochloric acid had been added, the chlorine dioxide concentration was determined by means of an iodometric titration.

**Microbiological Analysis.** A 30 g sample of the carrot was aseptically taken and transferred into a sterile stomacher bag. A 10-fold dilution was made in peptone physiologic salt solution [PPS; 8.5 g/L NaCl and 1 g/L neutralized bacteriological peptone (Oxoid, Hampshire, U.K.)], and the sample was homogenized during 60 s by means of a stomacher Seward Laboratory blender 400 (UAC House, London, U.K.). Subsequently, a decimal dilution series in PPS was

made and appropriate dilutions were brought on pouring plates of plate count agar (PCA, Oxoid) to determine the total aerobic plate count. After an incubation period of 3 days at 30 °C, colony forming units (cfu) were counted.

**Sensorial Analysis.** Triangle tests were conducted to determine whether a difference in sensory quality existed between products due to the decontamination treatment used. The samples were treated and transferred into plastic, closed recipients at 4 °C until sensory evaluation. The sensory evaluation occurred in a taste room with isolated booths. For each test three coded samples, of which one differed from the two other samples, were presented to 18 trained panelists and equal numbers of the possible combinations were at random presented. Each panelist had to evaluate the samples for overall sensory quality and selected the odd sample. Furthermore, the panelists were asked why they chose a particular sample.

**Color Measurement.** Color measurements were conducted as an objective measurement for sensory analysis. Color measurements were performed by means of a portable spectrophotometer (CM-2500d, Konica Minolta Sensing, Osaka, Japan) running on Spectra Magix NX (Color Data software CM-S100w, Konica Minolta Sensing) software and expressed in the CIE  $L^*a^*b^*$  color space. The color is defined by three orthogonal coordinates.  $L^*$  is the lightness component, which ranges from 0 (black) to 100 (white). Parameters  $a^*$  (+red, -green) and  $b^*$  (+yellow, -blue) are the two chromatic components, which range from -120 to 120. A plastic 2 cm deep Petri dish was filled completely with carrots to prevent color interference of the underlying tabletop. The dish was closed and the measurement was performed by placing the instrument viewing port on the cover. To exclude variable cover surface conditions, the specular interference was included in the color measurement. Calibration of the instrument was performed by means of the measurement of a white tile (white calibration) and a zero calibration. The following settings were used: 100% UV; illuminant, D65; observer angle, 10°; measurement area, 8 mm. On each Petri dish 10 measurements were performed.

**Carotenoids.** Stock solutions of 12.5 mg of  $\beta$ -carotene, 17.5 mg of *trans*- $\beta$ -apo-8'-carotenal, and 1.0 mg of lutein in 100 mL of chloroform with 0.1% (w/v) BHT as antioxidant were made and were stored in amber bottles at -18 °C. Starting from the stock solutions, working standards were made to check weekly the concentration by measuring the absorbance at maximum wavelength. To prepare working solutions of  $\beta$ -carotene, *trans*- $\beta$ -apo-8'-carotenal, and lutein, respectively, 400  $\mu$ L, 400  $\mu$ L, and 1 mL were taken and evaporated under  $N_2$ . Subsequently, the  $\beta$ -carotene residue was dissolved in hexane to obtain  $0.50 \pm 0.03$  AU at 450 nm ( $E_{1\%} = 2592$ ). The lutein residue was dissolved in ethanol to obtain  $0.1 \pm 0.02$  AU at 445 nm ( $E_{1\%} = 2550$ ) and the *trans*- $\beta$ -apo-8'-carotenal residue in petroleum ether to obtain  $0.76 \pm 0.03$  AU at 457 nm ( $E_{1\%} = 2640$ ).

The extraction procedure was performed according to the method of Taungbodhitham et al. (18) with some minor modifications. During the analysis samples were protected from daylight by covering the glassware with aluminum foil. The carrot samples were mixed with a hand blender (Miniprimer, MR5000M, Braun, Krönberg, Germany), and then 1 g of carrots was analytically weighed in an Erlenmeyer flask. After the addition of *trans*- $\beta$ -apo-8'-carotenal as internal standard and 35 mL of ethanol/hexane (4:3, v/v), the sample was vigorously mixed by means of an Ultraturrax (Zipperer, Staufen, Germany). Furthermore, the samples were extracted under continuous agitation (Edmund Bühler, KS-15, Hechingen, Germany) for 15 min under  $N_2$  at room temperature. After a second mixing step, the mixture was filtered on a separation funnel. The folded filter was washed with 35 mL of ethanol/hexane mixture, twice with 12.5 mL of ethanol, and finally with 12.5 mL of hexane until a white residue remained on the filter. Then the filtrate was successively washed with 10% NaCl (w/v, twice) and with water (three times). After every washing step, the aqueous layer was discarded. Furthermore, the organic layer containing the carotenoids was dried over anhydrous sodium sulfate in a round-bottom flask and evaporated under reduced pressure at 40 °C until an oleoresin was obtained. Then, the resin was evaporated to dryness under nitrogen. The residue was redissolved with 3 mL of acetonitrile, sonicated (Transsonic 460/H, Elma Hans Schmidbauer, Singen, Germany) for 15 s, and filtered through a 0.45  $\mu$ m HPLC filter (13 mm,

PolyPure II Syringe Filters, Alltech Associates, Lokeren, Belgium) in an amber storage vial. Finally, appropriate dilutions of the carotenoid extracts were made in mobile phase, and HPLC analyses were performed immediately.

HPLC analyses for individual carotenoids were carried out with a Finnigan Surveyor HPLC system (Thermo Electron Corp., Waltham, MA) equipped with a quaternary pump delivery system with a degasser, a thermostatically controlled autosampler (set at 4 °C), a thermostatically controlled column oven (set at 30 °C), and a photodiode array (PDA) detector (set to collect data between 190 and 600 nm as well as to collect the individual chromatograms of 410, 450, and 470 nm). The HPLC system was running on ChromQuest V4.1 software. The individual carotenoids were separated on a reversed phase YMC-Pack C30 (250 mm  $\times$  4.6 mm i.d., S-5  $\mu$ m, YMC, Schermbek, Germany) column. A combination of two mobile phases was used: methanol/acetonitrile (90:10, v/v) (A) and ethyl acetate with 0.25% (v/v) triethylamine (B). The carotenoids were separated using a linear gradient starting from 100% A to reach 60% B at 25 min. This combination of solvents was maintained for 5 min to reach 100% A again at 35 min and was in turn maintained until 40 min. The used flow rate was 1 mL/min. The different carotenoids were identified by their absorbance spectra (wavelengths of maximum absorption and the spectral shape), their degree of polarity, which is linked to their elution time, and for lutein and  $\beta$ -carotene by comparison with the retention time of standards. The recovery of the internal standard (*trans*- $\beta$ -apo-8'-carotenal) was >87%. The results are expressed in micrograms per 100 g of fresh weight.

**Vitamin E.** Stock solutions of  $\alpha$ - and  $\gamma$ -tocopherol were made by adding 25 mg to 100 mL of hexane. The solutions were stored at -18 °C protected from light. A volume of 10 mL was taken and evaporated under  $N_2$ . The residue was redissolved with 10 mL of methanol. Subsequently, absorbance readings were taken for the  $\alpha$ -tocopherol standards at 292 nm ( $E_{1\%} = 76$ ) and for the  $\gamma$ -tocopherol standards at 298 nm ( $E_{1\%} = 91$ ). The concentration of the tocopherols was checked weekly by measuring the absorbance at the appropriate emission wavelength. The samples were mixed, and 5 g of sample was analytically weighed in an Erlenmeyer flask of 100 mL. Subsequently, 50 mL of hexane/ethanol (4:1, v/v) with 0.05% BHT was added.  $\gamma$ -Tocopherol was used as internal standard as  $\gamma$ -tocopherol is not present in carrots. The mixture was filtered on a separating funnel after a 15 min of extraction at room temperature. After the filter had been washed with 25 mL of hexane, the filtrate was washed twice with 10 mL of water. The organic layer was dried over  $Na_2SO_4$  and evaporated under reduced pressure at 50 °C until an oleoresin was obtained. Furthermore, the oleoresin was evaporated to dryness under nitrogen. The residue was redissolved with hexane, filtered through a 0.45  $\mu$ m HPLC filter (13 mm, nylon, syringe filters, Alltech Associates, Lokeren, Belgium) in a vial, and injected into the HPLC system. The injection volume was 20  $\mu$ L. HPLC analyses were performed with a HPLC 1100 series (Agilent, Waldbronn, Germany) composed of an online degasser (G1379), a quaternary pump (G1311) pumping at 1.0 mL/min, and an autosampler (G1329, 4 °C) with an ALS Thermostat II (G1330, 35 °C) connected with a fluorescence detector with the excitation wavelength set on 285 nm and the emission wavelength on 325 nm. The HPLC system was running on ChemStation Rev. A. 10.02 software. The tocopherols were separated on the normal phase Lichrosorb Si (KNA/25EC500LBJ, 250 mm  $\times$  4.6 mm i.d.  $\times$  1.4 in., 5  $\mu$ m, 60 Å, Knauer, Berlin, Germany) column. As mobile phase *n*-hexane containing 0.9% (v/v) isopropanol was used. The tocopherols ( $\alpha$ - and  $\gamma$ -) were identified by comparing their retention time and fluorescence spectrum with those of standards. The recovery of the internal standard ( $\gamma$ -tocopherol) was >85%. The results are expressed in micrograms per 100 g of fresh weight.

**Total Phenol Content.** To determine the total phenol content, the extraction procedure for total phenols described by Vinson et al. (19) was used. An analytically weighed portion (5 g) was diluted to 50 mL with 1.2 M HCl in 50% aqueous methanol, shaken for 2 h at 80 °C, and filtered. The extracts were performed in triplicates and stored for a maximum of 24 h at -20 °C. The total phenol content was determined with the Folin-Ciocalteu method. The procedure described by Waterman and Mole (20) was followed. An appropriate volume (10 mL)

of the filtrate was added to 5 mL of Folin–Ciocalteu reagent (10 times diluted) in a volumetric flask of 100 mL. After 6 min  $\pm$  10 s, 15 mL of 20% Na<sub>2</sub>CO<sub>3</sub> (w/v) was added. After dilution to the mark with distilled water and shaking, the mixture was allowed to react during 2 h at room temperature in the dark. Absorbance readings were taken against blank at 760 nm. The standard curve of total phenolics was made using gallic acid standard solution (0–400 mg/L) under the same procedure as above, except that an aliquot of 1 mL was used. Total phenolics in carrots were expressed as milligrams of gallic acid equivalents (GAE) per 100 g of fresh sample.

**Antioxidant Capacity.** The antioxidant capacity was determined by means of the ferric reducing antioxidant power (FRAP) technique (21). The extraction was based on the procedure of Lako et al. (22) with some minor modifications. About 5 g of homogenized carrot was weighed analytically and extracted with 22.5 mL of acetonitrile containing 4% (v/v) acetic acid. The mixture was mechanically shaken during 15 min under nitrogen and subsequently centrifuged for 15 min at 5000g at 10 °C, and the supernatant was retained. The extraction procedure was repeated and finalized by centrifuging for 15 min at 12000g at 10 °C. Both supernatants were collected and adjusted to 50 mL with 95% ethanol. Finally, the supernatants were filtered and stored under nitrogen at –80 °C.

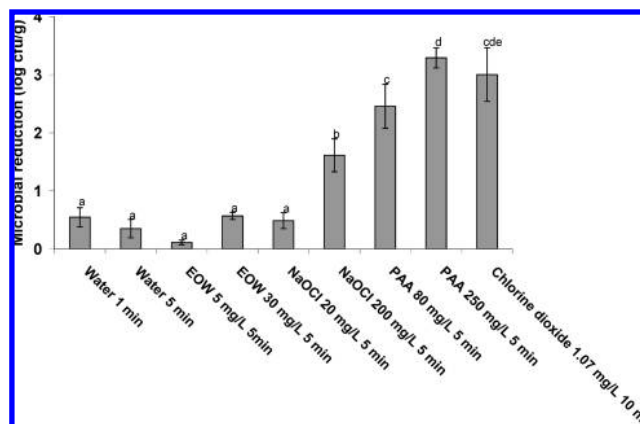
The FRAP was obtained by monitoring the absorbance change at 593 nm caused by the reduction of the Fe<sup>3+</sup>–TPTZ complex to the ferrous form at pH 3.6. The FRAP reagent was freshly prepared by mixing 25 mL of acetate buffer (300 mM, pH 3.6), 2.5 mL of TPTZ solution (10 mM) made in 40 mM HCl, and 2.5 mL of FeCl<sub>3</sub>·6H<sub>2</sub>O solution (20 mM) and was stored at 37 °C before use. Briefly, 100  $\mu$ L of the FRAP reagent or acetate buffer (blanks) was added to the samples or the standards. Absorbance was measured every minute during 20 min at 37 °C. FRAP values were obtained by comparing the absorbance change in the samples with those obtained from increasing concentrations of Fe<sup>3+</sup>.

All absorbance readings were performed by means of a microplate spectrophotometer (Benchmark Plus, Bio-Rad, Hercules, CA) using Microplate Manager Software 5.2.1. Results were expressed as micromoles of Fe<sup>2+</sup> per 100 g of fresh weight.

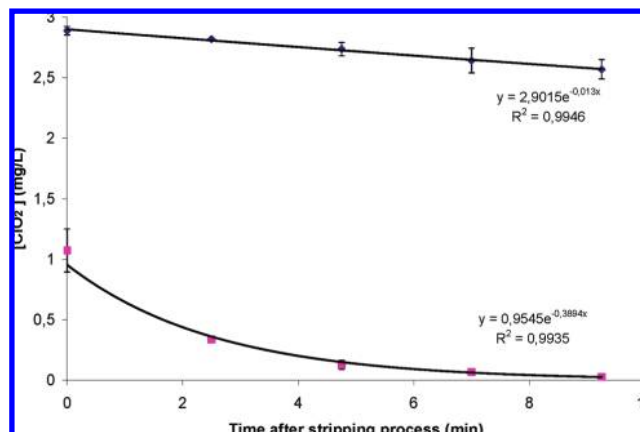
**Statistical Analysis.** Microbial reductions and nutrient contents were analyzed for significant differences ( $P < 0.05$ ) between the different series [control, water (reference treatment), low concentration of another disinfection agent, high concentration of another disinfection agent] and each decontamination agent (NaOCl, PAA, NEW, and ClO<sub>2</sub>) by using analysis of variance (one-way ANOVA). In case of significant differences, multiple comparison of means was established with the post hoc multiple-comparison Tukey test. All statistical analyses were performed using the software SPSS 7.0 for Windows (Insightful Corp., Seattle, WA). The results from the triangle tests were analyzed according to a one-sided binomial test. In the case of 18 assessors, the critical number of correct responses to obtain a statistically significant ( $\alpha = 0.05$ ) difference is 10 (23).

## RESULTS

**Effect of Decontamination Agents on the Initial Microbial Quality.** The microbial reductions of the total aerobic count obtained in grated carrots after a treatment with water (1 and 5 min), neutral electrolyzed oxidizing water (5 and 30 mg/L free chlorine), sodium hypochlorite (20 and 200 mg/L), peroxyacetic acid (80 and 250 mg/L), or gaseous chlorine (1.07 mg/L) dioxide are presented in **Figure 1**. On the basis of a literature screening and previous experiments conducted in our laboratory (results not shown), the concentrations of the decontamination agents were selected to cover the most frequently used concentration range of each decontamination agent. On the basis of the killing efficiency, a first series of treatments composed of water, neutral electrolyzed oxidizing water (5 and 30 mg free chlorine/L), and sodium hypochlorite (20 mg/L) inducing microbial reductions lower than 1 log cfu/g could be distinguished. Another group of treatments consisting of rinsing with sodium hypochlorite (200 mg/L), peroxyacetic acid (80 and 250 mg/L) and contact



**Figure 1.** Decontamination efficiency of different disinfection agents in fresh-cut carrots,  $n = 3$ . Error bars are standard deviations; a–e indicate statistical significance ( $\alpha = 0.05$ ).



**Figure 2.** Evolution of the concentration of gaseous chlorine dioxide in the empty chamber ( $\blacklozenge$ ,  $n = 3$ ) and during the treatment of 2 kg of grated carrots ( $\blacksquare$ ,  $n = 5$ ). Data points represent means, and error bars are standard deviations. Lines represent exponential regression.

with gaseous chlorine dioxide (1.07 mg/L) resulted in microbial reductions of 1.61, 2.46, 3.29, and 3.00 log cfu/g, respectively. Compared with the water rinsing step, the use of 200 mg/L sodium hypochlorite, peroxyacetic acid, and chlorine dioxide gas gave significantly higher reductions. The latter treatments gave an additional decontaminating effect that makes them useful disinfectants for grated carrots.

The evolution of the gaseous chlorine dioxide concentration in the empty treatment cabinet and during the treatment time of 2 kg of grated carrots is presented in **Figure 2**. Although chlorine dioxide gas losses were detected due to leakages of the treatment chamber, the decrease of the chlorine dioxide gas concentration in the empty cabinet is rather limited in a time period of 9.5 min after stripping. The initial chlorine dioxide gas concentration in the chamber filled with carrots was 1.07 mg/L. The chlorine dioxide gas concentration decreased exponentially when 2 kg of carrots was treated. When the area between the curve describing the decay of chlorine dioxide in the empty chamber and the curve describing the exponential decay in the filled chamber is taken as a measure for the chlorine dioxide gas consumption by the produce,  $90.42 \pm 1.12\%$  of the chlorine dioxide was consumed by the carrots.

### Effect of Decontamination Agents on the Sensory Quality.

To evaluate whether there is a difference in sensory quality between the water-treated samples and the samples treated with another disinfection agent or between different concentrations

**Table 1.** Results of Triangle Tests To Study the Effect of Different Decontamination Treatments on the Sensory Quality of Fresh-Cut Carrots ( $n = 18$ )

series	significant difference ( $\alpha = 0.05$ )?	comments about the odd sample
water—NaOCl 20 mg/L	no	
water—NaOCl 200 mg/L	yes	deviant odor and taste
NaOCl 20 mg/L—NaOCl 200 mg/L	no	
water — EOW $4 \pm 1$ mg/L	no	
water—EOW $30 \pm 2$ mg/L	yes	deviant odor and taste
EOW $4 \pm 1$ mg/L—EOW $30 \pm 2$ mg/L	yes	deviant odor and taste
water—PAA 80 mg/L	no	
water—PAA 250 mg/L	yes	deviant texture and taste
PAA 80 mg/L—PAA 250 mg/L	yes	deviant texture and taste
water—chlorine dioxide	no	

**Table 2.** Effect of a Peroxyacetic Acid (PAA) Treatment on Different Nutritional Parameters and Color (Mean  $\pm$  Standard Deviation)<sup>a</sup> of Fresh-Cut Grated Carrots

parameter	control	water	PAA (80 mg/L)	PAA (250 mg/L)
$\alpha$ -carotene <sup>a</sup> ( $\mu\text{g}/100$ g of fresh wt)	7767.66 $\pm$ 617.96 a	6877.08 $\pm$ 148.72 a	7274.78 $\pm$ 302.13 a	7917.54 $\pm$ 961.66 a
$\beta$ -carotene <sup>a</sup> ( $\mu\text{g}/100$ g of fresh wt)	17301.92 $\pm$ 1437.69 a	15086.81 $\pm$ 436.72 a	16018.76 $\pm$ 861.42 a	17554.97 $\pm$ 2405.94 a
$\zeta$ -carotene <sup>a</sup> ( $\mu\text{g}/100$ g of fresh wt)	1709.07 $\pm$ 113.16 a	1244.18 $\pm$ 102.80 b	1127.11 $\pm$ 69.85 b	1192.47 $\pm$ 123.60 b
lutein <sup>a</sup> ( $\mu\text{g}/100$ g of fresh wt)	213.63 $\pm$ 11.71 a	242.57 $\pm$ 48.41 a	268.12 $\pm$ 18.18 a	271.44 $\pm$ 17.27 a
total phenols <sup>a</sup> (mg of GAE/100 g of fresh wt)	34.54 $\pm$ 12.27 a	26.16 $\pm$ 5.17 a	20.49 $\pm$ 2.31 a	21.24 $\pm$ 3.10 a
$\alpha$ -tocopherol <sup>a</sup> ( $\mu\text{g}/100$ g of fresh weight)	941.48 $\pm$ 28.82 a	742.99 $\pm$ 127.51 b	464.42 $\pm$ 16.07 c	184.71 $\pm$ 18.68 d
FRAP antioxidant capacity <sup>a</sup> ( $\mu\text{mol}$ of $\text{Fe}^{2+}/100$ g of fresh wt)	14.64 $\pm$ 2.36 a	16.82 $\pm$ 2.61 a	12.28 $\pm$ 1.17 a	14.17 $\pm$ 2.63 a
$L^{*b}$	53.23 $\pm$ 1.16 a	55.16 $\pm$ 1.11 b	55.83 $\pm$ 1.20 b	55.30 $\pm$ 0.78 b
$a^{*b}$	22.46 $\pm$ 2.00 ab	21.58 $\pm$ 1.58 a	23.60 $\pm$ 1.99 bd	23.96 $\pm$ 1.36 cd
$b^{*b}$	23.61 $\pm$ 2.82 ab	22.56 $\pm$ 2.45 a	23.92 $\pm$ 2.31 ab	25.17 $\pm$ 1.83 b

<sup>a</sup> Values with different letters in one row show statistical significance ( $\alpha = 0.05$ ). <sup>b</sup>  $n = 3$ . <sup>c</sup>  $n = 30$ .

**Table 3.** Effect of a Sodium Hypochlorite (NaOCl) Treatment on Different Nutritional Parameters and Color (Mean  $\pm$  Standard Deviation)<sup>a</sup> of Fresh-Cut Grated Carrots

parameter	control	water	NaOCl (20 mg/L)	NaOCl (200 mg/L)
$\alpha$ -carotene <sup>a</sup> ( $\mu\text{g}/100$ g of fresh wt)	5130.39 $\pm$ 248.00 a	4432.72 $\pm$ 201.37 a	4894.66 $\pm$ 613.91 a	4499.33 $\pm$ 108.98 a
$\beta$ -carotene <sup>a</sup> ( $\mu\text{g}/100$ g of fresh wt)	12567.78 $\pm$ 594.77 a	10681.53 $\pm$ 729.39 a	11649.27 $\pm$ 1584.49 a	10614.03 $\pm$ 154.21 a
$\zeta$ -carotene <sup>a</sup> ( $\mu\text{g}/100$ g of fresh wt)	924.39 $\pm$ 51.80 a	593.55 $\pm$ 48.81 b	626.53 $\pm$ 73.41 b	437.08 $\pm$ 25.24 c
lutein <sup>a</sup> ( $\mu\text{g}/100$ g of fresh wt)	160.40 $\pm$ 59.27 a	157.46 $\pm$ 23.03 a	164.59 $\pm$ 50.27 a	166.00 $\pm$ 22.24 a
total phenols <sup>a</sup> (mg of GAE/100 g of fresh wt)	21.95 $\pm$ 0.31 a	15.51 $\pm$ 2.17 b	15.24 $\pm$ 1.10 b	16.35 $\pm$ 1.48 b
$\alpha$ -tocopherol <sup>a</sup> ( $\mu\text{g}/100$ g of fresh wt)	608.63 $\pm$ 22.17 a	491.83 $\pm$ 19.89 b	435.43 $\pm$ 44.79 b	251.99 $\pm$ 61.51 c
FRAP antioxidant capacity <sup>a</sup> ( $\mu\text{mol}$ of $\text{Fe}^{2+}/100$ g of fresh wt)	19.61 $\pm$ 0.02 a	17.61 $\pm$ 1.24 a	17.54 $\pm$ 0.08 a	17.61 $\pm$ 1.24 a
$L^{*b}$	52.90 $\pm$ 6.94 a	55.33 $\pm$ 0.85 bc	55.18 $\pm$ 0.93 ac	55.31 $\pm$ 1.26 ac
$a^{*b}$	23.33 $\pm$ 3.34 a	22.79 $\pm$ 1.49 a	22.14 $\pm$ 1.37 a	22.63 $\pm$ 1.60 a
$b^{*b}$	24.55 $\pm$ 4.07 a	23.41 $\pm$ 1.60 ac	22.67 $\pm$ 1.66 bc	22.90 $\pm$ 2.24 ac

<sup>a</sup> Values with different letters in one row show statistical significance ( $\alpha = 0.05$ ). <sup>b</sup>  $n = 3$ . <sup>c</sup>  $n = 30$ .

of one disinfection agent, triangle tests were conducted. For the liquid treatments, the lower concentrations of disinfection treatment, 20 mg/L for NaOCl, 80 mg/L for peroxyacetic acid, and NEW containing 3–5 mg/L free chlorine did not cause significant differences in sensory quality when compared with rinsing by water (**Table 1**). This is in contrast with the higher concentrations that induced a significant difference in sensory quality when compared with the water treatment. Panelists observed a change in texture when 250 mg/L peroxyacetic acid was used, whereas the typical chlorine odor of the chlorine containing disinfectants was not noted. When two different concentrations of one technique were compared, the sensory panel did not note a difference for sodium hypochlorite, whereas a significant difference was detected for peroxyacetic acid and neutral electrolyzed oxidizing water. Gaseous chlorine dioxide did not influence the sensory characteristics of grated carrots. Apparently, the effect of a disinfection agent on the sensory, fresh-like attributes of grated carrots is highly dependent upon the type of disinfectant and its concentration.

The color of a food product is an important fresh-like attribute for the consumer to evaluate the quality of the product. Several

disinfection agents possess strong oxidizing properties related with deleterious effects on the color of vegetables by inducing browning or bleaching of the vegetable tissue. To determine the effect of the previously mentioned decontamination agents on the color of grated carrots, color measurements were performed. The  $L^*$ ,  $a^*$ , and  $b^*$  values are presented in **Tables 2–5**. Rinsing with water induced in all of the experiments a significant increase of the  $L^*$  value, indicating a change to more white color components. The effect of the water treatment on the  $a^*$  and  $b^*$  value is not univocal. In three of the four vegetable batches (peroxyacetic acid, sodium hypochlorite, and electrolyzed oxidizing water) the  $a^*$  value did not change significantly. In the case of the fourth batch (chlorine dioxide) the  $a^*$  value decreased significantly after the water-rinsing step, indicating a shift to a less red color. In two of the four batches the  $b^*$  value did not change significantly, whereas in the other batches the  $b^*$  value decreased significantly, indicating a less yellow color. The peroxyacetic acid treatments had no additional influence on the  $L^*$  value when compared with the water treatment. When compared with both the control series and the water-rinsed series, the carrots treated with 250 mg/L peroxy-

**Table 4.** Effect of a Neutral Electrolyzed Oxidizing Water (EOW) Treatment on Different Nutritional Parameters and Color (Mean  $\pm$  Standard Deviation)<sup>a</sup> of Fresh-Cut Grated Carrots

parameter	control	water	EOW <sup>d</sup> (4 $\pm$ 1 mg/L)	EOW <sup>d</sup> (30 $\pm$ 2 mg/L)
$\alpha$ -carotene <sup>a</sup> ( $\mu$ g/100 g of fresh wt)	5502.28 $\pm$ 606.85 a	3945.61 $\pm$ 400.90 b	4350.21 $\pm$ 330.47 b	3895.85 $\pm$ 261.24 b
$\beta$ -carotene <sup>a</sup> ( $\mu$ g/100 g of fresh wt)	12573.33 $\pm$ 1765.93 a	8397.53 $\pm$ 1285.56 b	8948.90 $\pm$ 367.84 b	8611.56 $\pm$ 418.35 b
$\zeta$ -carotene <sup>a</sup> ( $\mu$ g/100 g of fresh wt)	1342.51 $\pm$ 92.52 a	1027.29 $\pm$ 121.08 b	967.55 $\pm$ 36.86 bc	744.70 $\pm$ 144.13 c
lutein <sup>a</sup> ( $\mu$ g/100 g of fresh wt)	163.34 $\pm$ 15.7 a	129.81 $\pm$ 14.11 a	162.02 $\pm$ 1.31 a	145.50 $\pm$ 30.80 a
total phenols <sup>a</sup> (mg of GAE/100 g of fresh wt)	32.44 $\pm$ 2.21 a	28.26 $\pm$ 4.70 a	26.04 $\pm$ 1.35 a	27.63 $\pm$ 2.41 a
$\alpha$ -tocopherol <sup>a</sup> ( $\mu$ g/100 g of fresh wt)	363.76 $\pm$ 51.93 a	394.68 $\pm$ 58.80 a	313.82 $\pm$ 18.84 a	297.35 $\pm$ 30.35 a
FRAP antioxidant capacity <sup>a</sup> ( $\mu$ mol of Fe <sup>2+</sup> /100 g of fresh wt)	10.56 $\pm$ 0.93 a	10.80 $\pm$ 0.18 a	11.29 $\pm$ 1.72 a	9.69 $\pm$ 3.88 a
L <sup>*b</sup>	53.90 $\pm$ 1.03 a	55.57 $\pm$ 1.09 b	55.44 $\pm$ 1.22 b	55.68 $\pm$ 0.99 b
a <sup>*b</sup>	23.29 $\pm$ 1.69 a	22.44 $\pm$ 1.81 a	23.09 $\pm$ 1.28 a	22.92 $\pm$ 1.40 a
b <sup>*b</sup>	24.23 $\pm$ 2.80 a	22.50 $\pm$ 2.08 b	23.52 $\pm$ 2.07 ab	23.20 $\pm$ 2.04 ab

<sup>a</sup> Values with different letters in one row show statistical significance ( $\alpha = 0.05$ ). <sup>b</sup>  $n = 3$ . <sup>c</sup>  $n = 30$ . <sup>d</sup> Free chlorine.

**Table 5.** Effect of a Gaseous Chlorine Dioxide Treatment on Different Nutritional Parameters and Color (Mean  $\pm$  Standard Deviation)<sup>a</sup> of Fresh-Cut Grated Carrots

parameter	control	water	chlorine dioxide
$\alpha$ -carotene <sup>a</sup> ( $\mu$ g/100 g of fresh wt)	4194.01 $\pm$ 218.51 a	3867.11 $\pm$ 78.20 a	3486.51 $\pm$ 70.08 b
$\beta$ -carotene <sup>a</sup> ( $\mu$ g/100 g of fresh wt)	10145.65 $\pm$ 485.54 a	8986.65 $\pm$ 136.03 b	8185.30 $\pm$ 137.39 c
$\zeta$ -carotene <sup>a</sup> ( $\mu$ g/100 g of fresh wt)	727.95 $\pm$ 29.52 a	618.28 $\pm$ 42.05 b	480.93 $\pm$ 14.12 c
lutein <sup>a</sup> ( $\mu$ g/100 g of fresh wt)	209.67 $\pm$ 18.49 a	172.31 $\pm$ 5.90 b	152.51 $\pm$ 4.32 b
total phenols <sup>a</sup> (mg of GAE/100 g of fresh wt)	34.15 $\pm$ 0.96 a	25.26 $\pm$ 1.24 b	27.53 $\pm$ 2.75 b
$\alpha$ -tocopherol <sup>a</sup> ( $\mu$ g/100 g of fresh wt)	516.74 $\pm$ 55.23 a	347.66 $\pm$ 14.16 b	370.75 $\pm$ 48.22 b
FRAP antioxidant capacity <sup>a</sup> ( $\mu$ mol of Fe <sup>2+</sup> /100 g of fresh wt)	12.68 $\pm$ 3.10 a	8.90 $\pm$ 1.05 a	10.33 $\pm$ 0.65 a
L <sup>*b</sup>	54.65 $\pm$ 0.86 a	55.23 $\pm$ 0.75 b	54.21 $\pm$ 0.69 a
a <sup>*b</sup>	24.54 $\pm$ 1.60 a	22.90 $\pm$ 1.48 b	21.52 $\pm$ 1.56 c
b <sup>*b</sup>	25.99 $\pm$ 2.23 a	23.86 $\pm$ 1.82 b	22.72 $\pm$ 2.33 b

<sup>a</sup> Values with different letters in one row show statistical significance ( $\alpha = 0.05$ ). <sup>b</sup>  $n = 3$ . <sup>c</sup>  $n = 30$ .

acetic acid showed an increased  $a^*$  value, indicating a more red color. A significantly higher  $b^*$  value was observed when carrots were treated with 250 mg/L peroxyacetic acid, indicating a shift to more yellow color components. When compared with the water series, a decontamination step with NaOCl influenced neither the  $L^*$  value nor the  $a^*$  value or  $b^*$  value. The same effect could be observed when neutral electrolyzed oxidizing water was used. Besides the effect of washing the grated carrots with water, no additional significant effect of an electrolyzed water treatment on color was stated. Although water rinsing induced an increase in the  $L^*$  parameter, showing a shift to more white color components, a subsequent treatment with chlorine dioxide decreased the  $L^*$  parameter again, indicating a more dark color. After a chlorine dioxide gas treatment, an additional decrease of the  $a^*$  parameter was observed, whereas the  $b^*$  value did not change significantly.

**Effect of Decontamination Agents on the Nutrient Content.** *Carotenoids.* From a nutritional point of view carrots are a major source of carotenoids in the human diet. The most predominant carotenoids in carrots and these evaluated in this study were  $\beta$ -carotene (10.1–17.3 mg/100 g of fresh weight) and  $\alpha$ -carotene (4.2–7.8 mg/100 g of fresh weight). Other evaluated carotenoids were  $\zeta$ -carotene (0.7–1.7 mg/100 g of fresh weight) and the xanthophyll lutein (160.40–213.63  $\mu$ g/100 g of fresh weight). Rinsing with water did not change in three of four batches the lutein content; in one batch a significant reduction of the lutein content was achieved. Although carotenoids are rather hydrophobic compounds, the effect of a water-rinsing step on carotene content varied between the different carrot batches and was dependent on the type of carotene. In three of the four batches,  $\alpha$ - and  $\beta$ -carotene content did not change after washing with water, whereas in one batch a significant reduction of both carotene contents was achieved. Rinsing grated carrots with water reduced the  $\zeta$ -carotene content

in all batches. The studied decontamination agents did not have an additional influence on the lutein content (**Tables 2–5**). Besides the reduction of the carotene content due to water rinsing, peroxyacetic acid and NaOCl as well as neutral electrolyzed oxidizing water did not cause significant reductions of the  $\alpha$ - and  $\beta$ -carotene content. In contrast with the liquid treatments, gaseous chlorine dioxide reduced both the  $\alpha$ - and  $\beta$ -carotene content by, respectively, 9.8 and 8.9%. The use of peroxyacetic acid (80 and 250 mg/L) did not change the  $\zeta$ -carotene content. The impact of NaOCl and electrolyzed oxidizing water on the  $\zeta$ -carotene content was dependent on the used concentration, where 20 mg/L NaOCl and electrolyzed oxidizing water containing 5 mg/L free chlorine did not change the  $\zeta$ -carotene content, higher concentrations of 200 mg/L NaOCl and electrolyzed oxidizing water containing 30 mg/L free chlorine reduced the  $\zeta$ -carotene content significantly with respect to the water-rinsed series. A similar result was obtained after a treatment with gaseous chlorine dioxide, for which an additional loss from 618.28 to 480.93  $\mu$ g/100 g of fresh weight was established.

*Total Phenols.* The effect of a decontamination process on the total phenol content of grated carrots is presented in **Tables 2–5**. Apart from the leaching effect, the respective decontamination agents, notwithstanding the concentration, did not have an influence on the total phenol content of grated carrots.

*$\alpha$ -Tocopherol.* Carrot samples were tested for the presence of tocopherols. By combining the retention time of tocopherol standards and their fluorescence spectra, only  $\alpha$ -tocopherol was retrieved. No other tocopherols were present in carrots in detectable amounts. In three of the four carrot batches a rinsing step with water during 5 min already reduced the initial  $\alpha$ -tocopherol content by 19.2–32.7%. Rinsing with both neutral electrolyzed oxidizing water and gaseous chlorine dioxide did not cause additional losses in  $\alpha$ -tocopherol content. The most

distinct losses were determined when grated carrots were rinsed with peroxyacetic acid. A treatment with 80 mg/L peroxyacetic acid during 5 min decreased the  $\alpha$ -tocopherol content by 37.5% when compared with the water-washed samples, whereas 250 mg/L peroxyacetic acid caused a reduction of 75.1% when compared with the water series. Next to peroxyacetic acid, 200 mg/L NaOCl also reduced the  $\alpha$ -tocopherol content significantly, but 20 mg/L NaOCl did not have any effect. Apparently, the concentration of sodium hypochlorite played a role in the presence of  $\alpha$ -tocopherol losses.

**Total Antioxidant Capacity.** The effect of a decontamination step on individual antioxidants was determined, as was its effect on the total antioxidant capacity. The combination of the individual antioxidants tested, other antioxidants, and the synergistic action between the different antioxidants are included in the total antioxidant capacity. The antioxidant capacity was measured by means of the FRAP procedure. Disinfecting grated carrots with 80 and 250 mg/L peroxyacetic acid did not influence the FRAP antioxidant capacity (**Table 2**). Besides the mechanical effect of washing the grated carrots, neither the use of NaOCl and neutral electrolyzed oxidizing water nor contact with gaseous chlorine dioxide caused significant reductions in total antioxidant capacity measured by the FRAP method (**Tables 3–5**).

## DISCUSSION

In this research the effect of decontamination agents on a wide range of important quality parameters including microbiology, organoleptic properties, and nutrient content was examined. All of these characteristics are relevant to the evaluation of the effectiveness of a decontamination step in fresh-cut carrots. Contrary to the rather limited reported information about the effect of a decontamination step with the tested agents on the nutrient content of fresh-cut carrots, several reports deal with the effect on the microbiological quality. Martín-Diana et al. (24) reported that the use of NaOCl (120 mg/L available chlorine, pH 8.0, room temperature, 5 min) caused a significant reduction of about 1 log cfu/g, which is similar to our results. Washing ungrated carrots with cold (4 °C) and warm (50 °C) chlorinated water (200 mg/L, pH 8.0) reduced the aerobic mesophilic count by, respectively, 1.7 and 2.3 log cfu/g (12). These reductions are higher than the ones achieved here, probably because of the higher available chlorine concentration. With a similar experimental setup for the production and treatment with 1.33 mg/L gaseous chlorine dioxide, Gómez-López et al. (13) reduced the mesophilic aerobic count by 1.88 cfu/g. In this study a microbial reduction of 3 log cfu/g could be obtained when a maximum chlorine dioxide gas concentration of 1.07 mg/L was used. The cause for this significantly better reduction is probably the choice for a rotating treatment chamber instead of a static chamber, which makes it possible to distribute the gas evenly over the produce. The discrepancy between the initial chlorine dioxide gas concentration in the empty and the filled chambers can be explained by the fact that the initial gas concentration was determined after the stripping process of 30 s (**Figure 2**). In the case of the filled chamber, the carrots instantly start to consume the chlorine dioxide. Ruiz-Cruz (25) evaluated washing with 200 ppm of NaOCl (pH 6.5) and 40 ppm of peroxyacetic acid during 2 min for their usefulness to decontaminate shredded carrots. These treatments caused a significant reduction of total aerobic count on day 0 when compared with the untreated carrots. Electrolyzed oxidizing water treatment with 20 ppm of available chlorine reduced the microbial load 0.4–0.7 log cfu/g on the surface of carrot slices when compared with water treatment (26).

Apparently, some of the disinfection treatments had beneficial effects on the microbial quality of grated carrots, although these effects were influenced by the large surface area in a mass of sample, which is in this case 0.27 m<sup>2</sup>/100 g of carrot sticks. The greater the degree of processing or size reduction, the greater the exposed interior tissue and the larger the cellular fluid exudation will be. Chlorine is known to be depleted by the reaction with organic matter. This is confirmed by the limited decontamination efficiency of those treatments with the lowest free chlorine concentrations (5 mg/L EOW, 30 mg/L EOW, and 20 mg/L NaOCl).

Other aspects of carrot quality are nutrient content and sensory attributes and color. In this paper data about the carotenoid content, the  $\alpha$ -tocopherol content, the total phenol content, and the antioxidant capacity of disinfected grated carrots were collected. Because of practical limitations it was not possible to perform the different analyses on the same carrot batch. Therefore, several batches of carrots were bought at a wholesale business. With respect to the nutrient content of the untreated carrots, a large variability could be observed. The content of nutrients (carotenoids, polyphenols, and  $\alpha$ -tocopherol) and subsequently the antioxidant capacity are affected by a wide range of factors such as the growing conditions and the growing season, the soil, genetic factors, the degree of ripeness, etc. (27). To encounter the problems of variability in nutrient content and to be able to quantify the eventual effects of a decontamination step, every experiment contained a control series (peeled and grated) and a water series (peeled, grated, and washed with potable water). Subsequently, the experiment was completed with a series treated with a specific disinfectant. Besides the natural variability in nutrient contents, the effect of a water-rinsing step on the nutrient content differed over the different carrot batches, probably due to a difference in initial texture of the carrots. Furthermore, the combination of a large contact area between the grated carrots and the rinsing solution and the rupture of cell membranes by minimal processing induced a decrease in nutrient content. Although not always significant,  $\alpha$ - and  $\beta$ -carotene content showed a decreasing trend after washing with water. This was confirmed by an increased  $L^*$  value indicating a shift to more white color components. Carotenoid content is one of the most relevant parameters in the to evaluation of sanitizers for their effect on the nutritional quality of fresh-cut carrots. In our study  $\beta$ -carotene was the most predominant identified carotenoid, followed by  $\alpha$ -carotene,  $\zeta$ -carotene, and lutein. Their contents in the untreated carrot samples varied between 10.1 and 17.30 mg/100 g, between 4.2 and 7.8 mg/100 g, between 0.7 and 1.7 mg/100 g, and between 160.40 and 213.63  $\mu$ g/100 g for  $\beta$ -carotene,  $\alpha$ -carotene,  $\zeta$ -carotene, and lutein, respectively. These values were consistent with those reported in the literature and were in the range of normal fluctuation, which is due to different cultivars, soils, fertilizers, climate, degree of ripeness, and storage condition before purchase (27).

Besides the part of the carotenoids that leached to the wash water, the liquid decontamination agents did not cause a reduction of the carotenoid content. Only a limited number of reports also evaluated the effect of different chemical decontamination treatments on the quality aspects of carrots. Of the nutrients analyzed in this study carotenoids (typical orange color) and phenols (role in enzymatic browning) can influence the color of carrots. Because the phenol content did not change after decontamination, the main changes in color are due to a change in the carotenoid content. Martín-Diana et al. (24) reported an increased luminosity when sliced carrots were treated with

chlorine (120 ppm, pH 8.0), due to its bleaching effect on tissue and an increased  $a^*$  parameter during storage because of browning. Contrary to the previous paper, the use of chlorinated water (200 ppm, 2 min) at both 4 and 50 °C did not cause significant changes in the whiteness index, a measure for white development on the carrot surface, or the chroma value, a measure for the saturation of the color, of shredded carrots (12). Also in this study, no significant effect of a sodium hypochlorite treatment on the color of grated carrots was observed, which corresponds with the lack of carotenoid loss due to the treatment. When fresh-cut carrots were treated with 150 ppm of NaOCl (pH 9.3) during 5 min, a carotenoid loss of 30% was obtained, which was similar to the loss caused by a treatment with potable water (28). According to Gómez-López et al. (13), the use of chlorine dioxide gas did not influence the sensorial attributes of grated carrots, which corresponds with our results. However, Sy et al. (7) reported that a treatment with 1.4 mg/L ClO<sub>2</sub> for 6.4–10.5 min at 79–84% relative humidity revealed a slight whitening in color, and consequently ratings assigned by a sensory panel for appearance, color, aroma and overall quality were significantly less good than the ratings for the untreated carrots. When compared with this study, the more pronounced deleterious effect on sensory quality could be due to the higher chlorine dioxide concentration (1.4 mg/L) used during the entire contact time, whereas in our case after 5 min, only 0.14 mg/L chlorine dioxide was present in the chamber due to its exponential decrease. Contrary to the liquid agents, gaseous chlorine dioxide has the most pronounced effect on carotenoid concentrations and the  $a^*$  value, which corresponds to a shift to a less red color. Gas will more easily penetrate into cut edges, cavities, and other irregularities than the other tested liquid agents, resulting in a more probable reactivity of chlorine dioxide against carotenoids. For similar reasons chlorine dioxide gas gave promising results in killing efficiency toward the total plate count of grated carrots. Izumi (26) reported that electrolyzed water (15–50 ppm of available chlorine) did not affect the surface color of carrot slices, which is similar to our results. The  $\beta$ -carotene content in carrots treated with acidic electrolyzed water (pH 2.5, 42.3 ppm free chlorine) for 10 min was decreased by about 30% but was not significantly different from the content in carrots soaked in tap water (28).

The change in nutrient content, more specifically the carotenoid content, could be related with a change in the color parameters ( $L^*$ ,  $a^*$ , and  $b^*$ ). However, panelists did not indicate a difference between water-washed carrots and carrots treated with a decontamination agent on the basis of a change in color. If the panelists could detect a significant difference, they indicated their choice for the odd sample with other sensory attributes such as texture, taste, and odor. Apparently the change in color due to carotenoid loss after a decontamination treatment was not pronounced enough to be detected during sensory evaluation.

In our study  $\xi$ -carotene was the least stable carotenoid during a decontamination process. Nevertheless, the losses of  $\xi$ -carotene due to decontamination have to be put into perspective, because the intake of  $\alpha$ -carotene and  $\beta$ -carotene is, from a nutritional point of view, far more important. Until now only two studies have dealt with the effect of sanitation procedures on initial total carotene content ( $\alpha$ - and  $\beta$ -carotene) of fresh-cut carrot and the content during storage (25, 28). Although there is no significant difference in initial carotene content between treated and untreated samples, carotene contents decreased continuously during storage to different extents among treatments. Carrots washed with acidified sodium chlorite (250 ppm) retained higher

carotene content compared with carrots washed with sodium hypochlorite (200 ppm) and peroxyacetic acid (40 ppm) (25). When 5 mm tomato slices were dipped into 0.34, 0.68, and 1.36% H<sub>2</sub>O<sub>2</sub> solutions for 1 min, the lycopene content was decreased significantly when compared with the contents of the control after 1 day (29). Although the peroxyacetic acid sanitizing solution also contains H<sub>2</sub>O<sub>2</sub>, we could not observe an effect on carotenoid content, possibly due to the lower concentrations, 0.12 and 0.37% instead of 0.34, 0.68, and 1.36% H<sub>2</sub>O<sub>2</sub>, and the different kind of fresh-cut product, carrot instead of tomato (28).

Apart from the decrease in total phenol content due to leaching to the washing water, the different decontamination treatments had no additional significant effect on the total phenol content of fresh-cut carrots. Similarly, soluble phenols were removed from the cut surfaces of sliced green peppers after washing with distilled water (30). In this paper total phenol content was determined by means of the spectrophotometric Folin–Ciocalteu method, which is sensitive for interfering compounds such as reducing sugars and proteins (31). Furthermore, reaction products, which can be formed as a consequence of the decontamination process and decrease the phenol content, can also interfere in the Folin–Ciocalteu assay (32). Of the broad family of phenolic compounds, carrots mainly contain hydroxycinnamic acids and their derivatives. Among them, chlorogenic acid was a major hydroxycinnamic acid, representing about 40–60% of total phenolic compounds detected in different carrot tissues (33).

In general, the impact of the various disinfection treatments on the total antioxidant capacity was not significant, although significant reductions of individual antioxidants were observed. The contrasting results can probably be explained by the different methods of extraction. The extraction solvent to determine the total antioxidant capacity was a rather hydrophilic mixture containing acetonitrile, acetic acid, and water, whereas for the extraction of single antioxidants more hydrophobic extraction solvents were used. Apparently, antioxidant and radical scavenging activities in carrots are mainly determined by the phenolics (33). Other antioxidants present such as carotenoids and vitamin E have a lower capacity to react with Fe<sup>3+</sup>, probably due to the rather hydrophilic extraction solution and the aqueous system in which the FRAP assay was conducted, as was described for other antioxidant assays (34, 35). Whereas the effect of some decontamination treatments on the  $\alpha$ -tocopherol content was most pronounced, the effect of the different sanitizers on the antioxidant capacity was rather limited.

Next to the reference treatment and the traditionally used sodium hypochlorite, the use of other and more innovative decontamination agents such as neutral electrolyzed oxidizing water, peroxyacetic acid, and gaseous chlorine dioxide opens perspectives as sanitizers in the fresh-cut produce industry. On the condition of an optimized treatment, the microbial quality of grated carrots could be improved without negatively influencing the sensorial attributes or the nutritional value of the product.

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#### LITERATURE CITED

- (1) Ragaert, P.; Verbeke, W.; Devlieghere, F.; Debevere, J. Consumer perception and choice of minimally processed vegetables and packaged fruits. *Food Qual. Pref.* **2004**, *15*, 259–270.



- (2) Beuchat, L. R.; Ryu, J. H. Produce handling and processing practices. *Emerg. Infect. Dis.* **1997**, *3*, 459–465.
- (3) CSPI, Center for the Science in Public Interest, Outbreak Alert! Washington, [http://www.cspinet.org/foodsafety/outbreak\\_alert.pdf](http://www.cspinet.org/foodsafety/outbreak_alert.pdf), 2007.
- (4) Adams, M. R.; Hartley, A. D. Factors affecting the efficacy of washing procedures used in the production of prepared salads. *Food Microbiol.* **1989**, *6*, 69–77.
- (5) Richardson, S. D.; Thruston, A. D.; Caughran, T. V.; Collette, T. W.; Patterson, K. S.; Lykins, B. W. Chemical by-products of chlorine and alternative disinfectants. *Food Technol.* **1998**, *52*, 58–61.
- (6) Nieuwenhuijsen, M. J.; Toledano, M. B.; Elliott, P. Uptake of chlorination by-products; a review and a discussion of its implications for exposure assessment in epidemiological studies. *J. Exp. Anal. Env. Epid.* **2000**, *10*, 586–599.
- (7) Sy, K. V.; Murray, M. B.; Harrison, M. D.; Beuchat, L. R. Evaluation of gaseous chlorine dioxide as a sanitizer for killing *Salmonella*, *Escherichia coli* O157:H7, *Listeria monocytogenes*, and yeasts and molds on fresh and fresh-cut produce. *J. Food Prot.* **2005**, *68*, 1176–1187.
- (8) Gonzalez, R. J.; Luo, Y. G.; Ruiz-Cruz, S.; McEvoy, J. L. Efficacy of sanitizers to inactivate *Escherichia coli* O157:H7 on fresh-cut carrot shreds under simulated process water conditions. *J. Food Prot.* **2004**, *11*, 2375–2380.
- (9) Uyttendaele, M.; Neyts, C.; Vanderswalmen, H.; Notebaert, E.; Debevere, J. Control of *Aeromonas* on minimally processed vegetables by decontamination with lactic acid, chlorinated water, or thyme essential oil solution. *Int. J. Food Microbiol.* **2004**, *90*, 263–271.
- (10) Ruiz-Cruz, S.; Acedo-Félix, E.; Díaz-Cinco, M.; Islas-Osuna, M. A.; González-Aguilar, G. A. Effect of sanitizers in reducing *Escherichia coli* O157:H7, *Salmonella* spp. and *Listeria monocytogenes* populations on fresh-cut carrots. *Food Control* **2007**, *18*, 1383–1390.
- (11) Brinez, W. J.; Roig-Sagues, A. X.; Herrero, M. M. H.; Lopez-Pedemonte, T.; Guamis, B. Bacterial efficacy of peracetic acid in combination with hydrogen peroxide against pathogenic and non pathogenic strains of *Staphylococcus*, spp., *Listeria* spp. and *Escherichia coli*. *Food Control* **2006**, *17*, 516–521.
- (12) Klaiber, R. G.; Baur, S.; Wolf, G.; Hammes, W. P.; Carle, R. Quality of minimally processed carrots as affected with warm water washing and chlorination. *Innovative Food Sci. Emerging Technol.* **2005**, *6*, 351–362.
- (13) Gómez-López, V. M.; Devlieghere, F.; Ragaert, P.; Debevere, J. Shelf-life extension of minimally processed carrots by gaseous chlorine dioxide. *Int. J. Food Microbiol.* **2007**, *116*, 221–227.
- (14) Dillard, C. J.; German, J. B. Phytochemicals: nutraceuticals and human health. *J. Food Sci. Agric.* **2000**, 1744–1756.
- (15) Lee, J.; Koo, N.; Min, D. B. Reactive oxygen species, aging and oxidative nutraceuticals. *Compr. Rev. Food Sci. Food Saf.* **2004**, *3*, 21–33.
- (16) O'Neill, M. E.; Carroll, Y.; Corridan, B.; Olmedilla, B.; Granado, F.; Blanco, I.; Van den Berg, H.; Hiniger, I.; Rousell, A. M.; Chopra, M.; Southon, S.; Thurnham, D. I. A European carotenoid database to assess carotenoid intakes and its use in a five-country comparative study. *Br. J. Nutr.* **2001**, *85*, 499–507.
- (17) World Health Organisation Europe (WHO). Food based dietary guidelines in the WHO European region; WHO Regional Office for Europe, Copenhagen, Denmark, 2003.
- (18) Taungbodhitham, A. K.; Jones, G. P.; Wahlqvist, M. L.; Briggs, D. R. Evaluation of extraction method for the analysis of carotenoids in fruits and vegetables. *Food Chem.* **1998**, *63*, 577–584.
- (19) Vinson, J. A.; Hao, Y.; Su, X.; Zubik, L. Phenol antioxidant quantity and quality in foods: vegetables. *J. Agric. Food Chem.* **1998**, *46*, 3630–3634.
- (20) Waterman, P. G.; Mole, S. *Analysis of Phenolic Plant Metabolites*; Blackwell Scientific Publications: Oxford, U.K., 1994.
- (21) Pellegrini, N.; Serafini, M.; Colombi, B.; Del Rio, D.; Salvatore, S.; Bianchi, M.; Brighenti, F. Total antioxidant capacity of plant foods, beverages and oils consumed in Italy assessed by three different in vitro assays. *J. Nutr.* **2003**, *133*, 2812–2819.
- (22) Lako, J.; Trenerry, V. C.; Wahlqvist, M.; Wattanapenpaiboon, N.; Sotheeswaran, S.; Premier, R. Phytochemical flavonols, carotenoids and antioxidant properties of a wide selection of Fijian fruit, vegetables and other readily available foods. *Food Chem.* **2007**, *101*, 1727–1741.
- (23) Meilgaard, M. C.; Civille, G. V.; Carr, B. T. *Sensory Evaluation Techniques*, 4th ed.; CRC Press: Boca Raton, FL, 2006.
- (24) Martín-Diana, A. B.; Rico, D.; Barry-Ryan, C.; Frías, J. M.; Mulcahy, J.; Henehan, G. T. M. Comparison of calcium lactate with chlorine as a washing treatment for fresh-cut lettuce and carrots: quality and nutritional parameters. *J. Sci. Food Agric.* **2005**, *85*, 2260–2268.
- (25) Ruiz-Cruz, S.; Islas-Osuna, M. A.; Sotelo-Mundo, R. R.; Vázquez-Ortiz, F.; González-Aguilar, G. A. Sanitation procedure affects biochemical and nutritional changes of shredded carrots. *J. Food Sci.* **2007**, *2*, S146–S152.
- (26) Izumi, H. Electrolyzed water as a disinfectant for fresh-cut vegetables. *J. Food Sci.* **1999**, *64*, 536–539.
- (27) Müller, H. Determination of the carotenoid content in selected vegetables and fruit by HPLC and photodiode array detection. *Z. Lebensm. Unters. Forsch.* **1997**, *204*, 88–94.
- (28) Koseki, S.; Itoh, K. The effect of acidic electrolysed water on the quality of cut vegetables. *J. Jpn. Soc. Food Sci. Technol.* **2001**, *48*, 365–369.
- (29) Kim, H.-J.; Fonseca, J. M.; Kubota, C.; Choi, J.-H. Effect of hydrogen peroxide on quality of fresh-cut tomato. *J. Food Sci.* **2007**, *72*, S463–S467.
- (30) Toivonen, P. M. A.; Stan, S. The effect of washing on physico-chemical changes in packaged, sliced green peppers. *Int. J. Food Sci. Technol.* **2004**, *39*, 43–51.
- (31) Escarpa, A.; González, M. C. Approach to the content of total extractable phenolic compounds from different food samples by comparison of chromatographic methods. *Anal. Chim. Acta* **2001**, *427*, 119–127.
- (32) Nicoli, M. C.; Anese, M.; Parpinel, M. Influence of processing on the antioxidant properties of fruit and vegetables. *Trends Food Sci. Technol.* **1999**, *10*, 94–100.
- (33) Zhang, D.; Hamauzu, Y. Phenolic compounds and their antioxidant properties in different tissues of carrots (*Daucus carota* L.). *J. Food Agric. Environ.* **2004**, *2*, 95–100.
- (34) Soares, D. G.; Andrezza, A. C.; Salvador, M. Sequestering ability of butylated hydroxytoluene, propyl gallate, resveratrol, and vitamins C and E against ABTS, DPPH, and hydroxyl free radicals in chemical and biological systems. *J. Agric. Food Chem.* **2003**, *51*, 1077–1080.
- (35) Pellegrini, N.; Colombi, B.; Salvatore, S.; Brenna, O. V.; Galaverna, G.; Del Rio, D.; Bianchi, M.; Bennett, R. N.; Brighenti, F. Evaluation of antioxidant capacity of some fruit and vegetable foods: efficiency of extraction of a sequence of solvents. *J. Sci. Food Agric.* **2007**, *87*, 103–111.

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